BIOGRAPHICAL SKETCH

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NAME: Ronald N. Germain

eRA COMMONS USER NAME (credential, e.g., agency login): rgermain

POSITION TITLE: Chief, Laboratory of Immune System Biology, NIAID, NIH

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Brown University, Providence, RI	Sc. B.	06/70	Biology
Brown University, Providence, RI	Sc. M.	06/70	Immunology
Harvard University, Boston, MA	Ph. D.	06/76	Immunology
Harvard University, Boston, MA	M.D.	06/76	Medicine

A. Personal Statement

I have been conducting immunological research since high school, beginning with studies testing whether adult thymic grafts would rescue neonatal animals from lethal GvH disease. During college I continued my immunological research, culminating in the simultaneous award of a Master's and Bachelor's degrees in 4 years. I was one of the first 2 students in the Harvard Immunology MSTP program, completing the combined MD PhD in six years and moving directly onto the Harvard faculty in 1976. Over the past 40 years, I have led a research group that has made key contributions to understanding MHC molecule structure-function, the cell biology of antigen processing and presentation, how T cells signal in response to peptide-MHC ligands, and over the last 15 years, pioneering the use of in vivo dynamic and multiplex static imaging to uncover new insights into how the innate and adaptive immune systems are organized and operate in situ. I have also played a major role in bringing computational modeling into use in modern immunology and in applying systems biology approaches to basic and clinical research on the immune system. I have trained more than 70 postdoctoral fellows, many of whom have gone on to achieve academic distinction as independent investigators, including Sir Robert Lechler, Caetano Reis e Sousa, Takashi Saito, Joaquin Madrenas, Hai Qi, Andrea Sant, Franca Ronchese, and many others.

B. Positions and Honors

Positions and Employment

1976-1977 Instructor, Pathology, Harvard Medical School and Intern, Pathology, Peter Bent Brigham Hosp.
1977-1980 Assistant Professor, Pathology, Harvard Medical School
1980-1982 Associate Professor, Pathology, Harvard Medical School
1981-1982 Guest Investigator, Laboratory of Molecular Genetics, NICHHD, NIH
1982-1987 Senior Investigator, Laboratory of Immunology, NIAID, NIH
1987-Date Chief, Lymphocyte Biology Section, Laboratory of Immunology, NIAID, NIH
1989-2002 Senior Executive Service, DHHS
1994-2011 Deputy Chief, Laboratory of Immunology, NIAID, NIH
2006-2011 Director, Program in Systems Immunology and Infectious Disease Modeling, NIAID, NIH
2008-Date Associate Director - Systems Biology and Technology, Trans-NIH Center for Human Immunology, Inflammation, and Autoimmunity [CHI]
2011-2018 Chief, Laboratory of Systems Biology and NIH Distinguished Investigator
2015-2018 Acting Chief, Laboratory of Immunology
2016-Date Director, NIAID-NCI Center for Advanced Tissue Imaging (CAT-I)
2018-Date Chief, Laboratory of Immune System Biology

Other Experience (abbreviated)
1992-Date Advisory Editor, Journal of Experimental Medicine
1997-Date Editorial board, Current Biology
2003-2006 Editor, Immunity
2006-Date Associate Editor, Immunity
2007-2009 Editorial Board, J. Clinical Investigation
2007 Co-organizer of first Keystone Symposium on "Imaging Immune Responses"
2008-2011 Editorial Board Wiley Interdisciplinary Reviews Systems Biology and Medicine
2009-Date Editorial board of Molecular Systems Biology
2009-Date Editorial Board, International Immunology
2011-Date Editorial Advisory Panel, Scientific Reports, Nature
2014-Date Board of Reviewing Editors, eLife
2015 Co-organizer of the first Keystone meeting on Systems Immunology

Honors and Awards (abbreviated; past 15 years)
Benacerraf Lecturer, Dana-Farber Cancer Center, Harvard Medical School, 2002
ISI Highly Cited Researcher, 2003
Ernst Schering Foundation Lecturer, 2004
Distinguished Lecturer, American Association of Immunologists, 2006
R.G.E. Murray Lecturer, Univ. of Western Ontario, 2006
Australasian Society for Immunology (ASI) Visiting Lecturer, 2007
Landsteiner Medal, Austrian Soc. Allerology and Immunology, 2008
Foreign Associate, EMBO, 2008
Blumenthal Lecturer, Univ. of Minn., 2009
Shraga Segal Memorial Lecturer, Ben-Gurion University, Israel, 2010
Sidney Leskowitz Memorial Lecturer, Tufts University Medical School, Boston, MA 2011
Mayberry Lecturer, Northwestern University School of Medicine, 2011
Mary Lou Clements-Mann Memorial Lecturer in Vaccine Sciences, National Foundation for Infectious Diseases, 2011
Ralph Wedgwood Lecturer, World Immunology Conference, New York, NY 2011
NIH Distinguished Investigator, 2011
Akeson Memorial Lecturer, Cincinnati Children's Hospital, Cincinnati, OH, 2011
Ishizaka Lecturer, La Jolla Inst. Allergy and Immunology, La Jolla, CA, 2011
Honorary Member, Scandinavian Society for Immunology, 2011
AAAS Fellow, 2012
IOM (now the NAM) member, 2013
AAI Meritorious Career Award, 2015
Medawar Centenary Lecturer, Francis Crick Mill Hill Laboratory, 2015
G. Burroughs Mider Lecturer, NIH, 2015
NIAID Outstanding Mentor Award, 2016
National Academy of Sciences member, 2016
William E . Paul Award for Excellence in Immunology and Cell Biology, Foundation for Primary Immunodeficiency Diseases, 2017

C. Contributions to Science

1. Analysis of MHC Class II molecule structure and function

Genetic data indicated that MHC encoded molecules played a key role in controlling T cell responses, and there was some understanding of the molecules at the protein level, but in the early 1980's the corresponding genes had yet to be cloned to allow detailed analysis of the organization, polymorphism, and functional attributes of these molecules. Working with Jonathan Seidman on a 'sabbatical' at NICHD, NIH, I contributed to the first direct cloning of a mouse class II gene. I then went on as a new tenured investigator at NIH to develop one of the top international programs in analysis of MHC Class II structure function using newly developed methods of site directed mutagenesis and gene transfection. Prior to the publication of the Bjorkman-Wiley MHC Class I structure, we reported a correct general description of MHC molecule organization based on our detailed mapping of the roles of polymorphic residues in controlling peptide presentation, TCR recognition, antibody

binding, and dimer assembly. We provided new insights into how MHC class II molecules bound peptide and the unexpected role of peptide in stabilizing the MHC class II structure. The latter observation led to a novel method for tracking the pathway of MHC class II molecule assembly and intracellular trafficking during maturation into a peptide-loaded, surface presented ligand for the TCR. Finally, we mapped the site of interaction of MHC class II molecules with the CD4 corrector.

Germain, R., Norcross, M., and Margulies, D.: Functional expression of a transfected murine class II MHC gene. *Nature*. 306: 190-194, 1983.
Ronchese, F., Schwartz, R.H., and Germain, R.N.: 1987. Functionally distinct subsites on a class II major histocompatibility complex molecule. *Nature*. 329: 254-256.
Braunstein, N.S., and Germain, R.N.: Allele-specific control of Ia surface expression and molecular conformation: Implications for a general model of Ia structure-function relationships. *Proc. Natl. Acad. Sci. U.S.A*. 84: 2921-2925, 1987.
Sadegh-Nasseri, S., and Germain, R.N.: A role for peptide in determining MHC class II structure. *Nature,* 353: 167-170, 1991.

2. The cell biology of MHC class II antigen processing and presentation

Having developed tools for understanding MHC class II molecule structure and function, the next important question in the field was how these molecules and MHC class I molecules acquired their peptide cargo. The first integrated model suggesting that MHC class I and Class II molecules bind peptides derived from proteins in different intracellular compartments was a synthetic piece I published in Nature in 1986. According to its inventor, DNA vaccination was developed as a direct result of the insights in this article, establishing the importance of this contribution to translation medicine. Based on our detailed understanding of the fine structure of MHC class II molecules, we went on to map interaction with the invariant chain and used this knowledge along with our demonstration of peptide stabilization of MHC class II dimers to track the maturation of MHC class II molecules as they moved through the cell and gained peptide cargo. Our map of antibody binding to MHC class II molecules allowed us to avoid a major error in the pulse chase studies published by other groups. As a result, our cell fraction studies have stood the test of time as the correct description of the route followed by MHC class II molecules during active processing. We also showed that MHC class II molecules bind large protein cargo rather than short peptides in endosomes and that invariant chain does not prevent short peptides destined for MHC class II binding from interacting with MHC class II molecules, all consistent with my 1986 Nature paper.

Germain, R.N.: The ins and outs of antigen processing and presentation. *Nature*. 322: 687-689, 1986.
Germain, R.N., and Hendrix, L.R.: MHC class II structure, occupancy, and surface expression determined by post-endoplasmic reticulum antigen binding. *Nature*. 353: 134-139, 1991.
Romagnoli, P., and Germain, R.N.: The CLIP region of invariant chain plays a critical role in regulating MHC class II folding, transport, and peptide occupancy. *J. Exp. Med*. 180, 1107-1113, 1994.
Castellino, F., Germain, R.N.: Extensive trafficking of newly synthesized MHC class II-invariant chain complexes in the endocytic pathway and appearance of peptide-loaded class II molecules in multiple compartments. *Immunity*. 2: 73-88, 1995.

3. Analysis and modeling of TCR signaling in response to peptide-MHC ligands

While the field made rapid progress in defining the ligands involved in activation of T cells, the recognition structure(s) remained obscure. With the cloning success of the Davis, Mak, and Tonegawa laboratories, the question became whether a single $\alpha\beta$ dimer truly accounted for the dual antigen and MHC specificity of T cells and how the TCR signaled upon interaction with its ligand. Having defined in detail the structure-function relationships of the peptide-MHC ligand for the TCR and the cell biology and antigen processing, we turned to an analysis of how these ligands interacted with the T cell receptor and the signals generated by this interaction. These analyses involved the first evidence using gene transfer methods that a single $\alpha\beta$ dimer could transfer both antigen and MHC class II specificity to another T cell, helping to validate the evidence that these recently identified polypeptides truly accounted for the dual specificity of T cells. Our studies also resulted in the co-discovery of peptide antagonists and provided important contributions to understanding how altered ligands affected T cell signaling and responses. We uncovered a novel set of opposing intracellular feedback pathways involved in TCR ligand discrimination, described how the tuning of these pathways accounted for important aspects of positive and negative selection of thymocytes, used these data to develop a mathematical model of TCR signaling, and employed this model in conjunction with some of the first single cell signaling assays to describe the heterogeneity of individual cell behaviors linked to fluctuations in gene/protein expression, a topic

of considerable current interest nearly a decade after our work on this topic. We also discovered the role of self-ligand in sustaining the sensitivity of T cells to foreign antigen.

Saito, T., Weiss, A., Miller, J., Norcross, M.A., and Germain, R.N.: Specific antigen-Ia activation of transfected human T cells expressing murine Ti □-human T3 complexes. *Nature* 325: 125-129, 1986.
Racioppi, L., Ronchese, F., Matis, L.A., and Germain, R. N.: Peptide-major histocompatibility complex class II complexes with mixed agonist/antagonist properties provide evidence for ligand-related differences in T cell receptor-dependent intracellular signaling. *J. Exp. Med.* 177:1047-1060, 1993.
Madrenas, J., Wange, R.L., Wang, J.L., Isakov, N., Samelson, L.E., and Germain, R.N.: ζ phosphorylation without ZAP-70 activation induced by TCR antagonists or partial agonists. *Science*. 267: 515-518, 1995.
Feinerman, O., Veiga, J., Dorfman, J.R., Germain, R.N., and Altan-Bonnet, G. Variability and robustness in T cell activation from regulated heterogeneity in protein levels. *Science*, 321:1081-4, 2008.

4. Development and application of advanced imaging methods for understanding the immune system in situ
Two decades of study of the molecules involved in T cell antigen recognition left open the question of how T cells interacted with antigen bearing cells in vivo during 'real' immune responses, how the immune system facilitates efficient interactions among rare cells, and the dynamics of immune cell movement and interaction in complex tissue environments. In 2002, three groups including my laboratory published the first reports of dynamic imaging of immune cells in complex tissue environments. Since that time, we have pioneered the application of 2-photon imaging to diverse tissues and cell types, developing an increasing detailed picture of how tissue micro-anatomy facilitates immunity and characterizing the behavior of different immune cells in vivo. Our work has provided many novel insights, ranging from the stable interactions of T cells with antigen-bearing dendritic cells, the migration of T cells along fibroblastic reticular cell fibers in lymph nodes, the role of chemokines in facilitating multicellular co-operation, the unexpected role of SAP and SLAM family members in lymphoid cell-lymphoid cell (e.g., T-B) interactions, the existence of a layered organization of cells promoting innate immunity within lymph nodes, the unexpectedly rare nature of cells showing cytokine production in pathogen rich sites such as granulomas, the role of TCR tuning via PD-1 in controlling the extent of effector T cell function in tissues, the S1P-mediated control of osteoclastogenesis, the molecular control of neutrophil swarming behavior in both infected and sterile lesions, and the role of multiple dendritic cells in controlling CD4-CD8 T cell interactions in viral responses. More recently, to overcome the limited multiplex capacity of such intravital imaging and the small tissue volumes involved, as well as to produce a method for detailed analysis of human samples, we developed a new static imaging method called Histo-cytometry that allows highly multiplex (14+ color) staining and quantitative analysis of cells in tissue samples. We have used this method to reveal the unexpected complex positioning of differing dendritic cell subsets in lymph nodes, the role of dendritic cell subset positioning in functional biology, and the importance of TCR and IL-2 signals within small defined clusters of cells in Treg control of autoimmune T cell responses. Most recently, we have developed a new clarification method that permits application of Histo-cytometry to 3D imaging of large tissue volumes, with very important implications for analysis of human biopsy samples – using 12-15 colors with samples 500 u thick provide 250 times the data typically acquired using a thin tissue section analyzed by conventional histochemistry. There is widespread pharmaceutical and biotech interest in partnering with my group to use these novel imaging methods for both pre-clinical and clinical studies of drug delivery and analysis of patients undergoing cancer immunotherapy, among other possible uses of the method.

Stoll, S., Delon, J., Brotz, T. M. and Germain, R. N.: Dynamic imaging of T cell-dendritic cell interactions in lymph nodes. *Science* 296: 1873-1876, 2002.
Castellino, F., Huang, A.Y.C., Altan –Bonnet, G., Stoll, S., Scheinecker, C., and Germain, R.N.: Chemokines enhance immunity by guiding naive $CD8^+$ T cells to sites of $CD4^+$ T cell-dendritic cell interaction. *Nature* 440:890-895, 2006.
Qi, H., Cannons, J., Schwartzberg, P., and Germain, R.N. SAP-controlled T-B interactions underlie the formation of germinal centres. *Nature,*455:764-9, 2008.
Gerner, M.Y., Kastenmuller, W., Ifrim, I., Kabat, J., and Germain, R.N. Histo-Cytometry: A method for highly multiplex quantitative tissue imaging analysis applied to dendritic cell subset microanatomy in lymph nodes. *Immunity*. 37:364-376, 2012.
Liu, Z., Gerner, M.Y., Van Panhuys, N., Levine, A.G., Rudensky, A.Y., and Germain, R.N. Immune homeostasis enforced by co-localized effector and regulatory T cells. *Nature*. 528:225- 30, 2015.

Eickhoff, S., Brewitz, A., Gerner, M.Y., Klauschen, F., Komander, K., Hemmi, H., Garbi ,N., Kaisho, T., Germain, R.N., and Kastenmüller ,W. Robust anti-viral immunity requires multiple distinct T cell-dendritic cell interactions. *Cell*. 162:1322-37, 2015.

Brewitz, A., Eickhoff, S., Dähling, S., Quast, T., Bedoui, S., Kroczek, R.A., Kurts, C., Garbi, N., Barchet, W., Iannacone, M., Klauschen, F., Kolanus, W., Kaisho, T., Colonna, M., Germain, R.N., and Kastenmüller W. $CD8_+$ T cells orchestrate pDC-$XCR1_+$ dendritic cell spatial and functional cooperativity to optimize priming. *Immunity* 46:205-219, 2017

Gerner, M.Y., Casey, K.A., Kastenmuller, W., and Germain, R.N. Dendritic cell and antigen dispersal landscapes regulate T cell immunity. *J Exp Med.* 214(10):3105-3122, 2017.

Li, W., Germain, R.N., and Gerner, M.Y. Multiplex, quantitative cellular analysis in large tissue volumes with clearing-enhanced 3D microscopy (C_e3D). *Proc Natl Acad Sci*. *USA* 114(35):E7321-E7330, 2017.

Mao, K., Baptista, A.P., Tamoutounour, S., Zhuang, L., Bouladoux, N., Martins, A.J., Huang, Y., Gerner, M.Y., Belkaid, Y., and Germain, R.N. Innate and adaptive lymphocytes sequentially shape the gut microbiota and lipid metabolism. *Nature* 554:255-259, 2018

My Bibliography URL: https://www.ncbi.nlm.nih.gov/pubmed/?term=germain+rn

D. Additional Information: Research Support and/or Scholastic Performance

My research is fully supported by the Intramural Research Program of the NIAID, NIAID